The effect of AQP4 mRNA interference on AQP4 expression in cerebral edema and the manifestation of diffusion-weighted imaging in rat brain

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Purpose

To investigate the effection of the AQP4 expression for cerebral edema after AQP4 mRNA interference(RNAi) and the diagnostic values of diffusion-weighted imaging(DWI).
Methods and Materials

One hundred and fourteen Wistar rats were divided into nineteen groups randomly: normal group, control group, ischemic groups MCAO and interference groups siRNA-AQP4 MCAO. Above all groups were divided into six sub-groups according to the different interval of time length: 15 min, 30 min, 1 h, 2 h, 4 h and 6 h, respectively (n=6 for each group), except for normal group (n=6). The rats in all groups were examined with DWI. The relative apparent diffusion coefficient (rADC) of the biggest hyperintensity signal layer on DWI were measured. After that the animals were sacrificed and perfused with the mixture solution consisting formalin. The brain tissue which corresponding to the DWI were examined with pathology, Real Time Fluorescence Quantitative Reverse Transcript Polymerase Chain Reaction (RT-FQ-PCR, Ex***Ct) and Western-blotting (D). The data was recorded as x±s and made statistics with SPSS13.0 software.
Results

1. DW-MRI and rADC values

The abnormal group showed no abnormal DWI signal at each time point and the mean rADC value of the right basal ganglia was (98.3 ± 1.6)%. There was no significant difference between the control and ischemic groups (P > 0.05). The control ischemic group showed a high DWI signal at 15 min after embolization (6/6) and the area of the high signal increased gradually with time. The rADC value decreased rapidly from (65.4 ± 8.3)% at 15 min to (40.5 ± 10.9)% at 2 h, and then decreased slowly from (40.2 ± 6.8)% at 4 h to (39.7 ± 7.9)% at 6 h. In the RNAi group, the rADC value decreased slowly without significant difference between baseline and 15 min (P > 0.05). The high DWI signal area was reduced significantly during the 30 min ~ 2 h period, and the rADC decreased slightly from (62.4 ± 9.5)% at 30 min to (55.4 ± 8.9)% at 2 h, and decreased further after 4 h ~ 6 h. There were significant differences between the ischemic and RNAi groups at 2 h (P < 0.01) and 4 h (P < 0.05) (Figure 1, 2, 3).

2. Pathological findings

The pathological changes in the ischemic groups were the same as the control group. 15 min after a middle cerebral artery occlusion in the ischemic group, it was observed under an optical microscope, a small amount of swollen glial cells with a rounded shape, increased volume, and lighter eosinophilic cytoplasm (Figure 4). Under an electron microscope, mitochondrial swelling, vacuole-like changes, expansion of the endoplasmic reticulum, nuclear swelling, and chromatin margination was observed. At 1 h, under a light microscope, the nucleus was darkly stained there was a partial eosinophilia change in the cytoplasm (red neurons), and further swelling of organelles was observed. Undisrupted membranes were observed under the electron microscope. At 2 h, nuclear condensation and glial cell swelling became more evident, a light transmission around the cell appeared, and cell interspaces decreased while the cell membrane remained intact. At 4 h endothelial cells appeared swollen, the spaces between blood vessels and cells expanded, glial cell membranes swelled and thickened, vasculars were compressed and deformed, and a light red mesh emerged in the tissue space which indicated vasogenic edema. Under the electron microscope, a partially ruptured cell membrane, caryolysis, chromatin margination, appearance of medullary structure within cytoplasm, and blood-brain barrier (BBB) damage was observed (Figure 5). At 6 h, the above change (vasogenic edema) continued to aggravate. The RNAi group showed significantly lower glial cell swelling at each time point compared with the control group with the largest difference observed at 2 h (Figure 6).

3. AQP4 mRNA expression

In the normal group AQP4 mRNA expression was detected in the basal ganglia. There was no significant difference in expression between the control and ischemic group (P >
In the control group AQP4 mRNA expression increased from 15 min (0.12 ± 0.02) up to 2 h of (0.47 ± 0.03); 4 h the trend increased at a slower rate. In the RNAi group, AQP4 mRNA expression increased slowly from (0.12 ± 0.02) at 15 min to (0.14 ± 0.02) at 2 h, and after 4 h it continued to increase from (0.24 ± 0.03) at 4 h to (0.31 ± 0.04) at 6 h. There were significant differences between the RNAi and ischemic group expression levels at each time point (P <0.01) (Figure 7) except at 15 min (P> 0.05).

4. AQP4 protein expression

The normal group showed a small amount of AQP4 protein expression in the basal ganglia. There was no significant difference between the control and ischemic group (P> 0.05). In the control group, AQP4 protein expression increased rapidly from (0.86 ± 0.02) at 15 min to (1.56 ± 0.08) at 2 h, and continued to increase slowly after 4 h. In the RNAi group, AQP4 protein expression increased slowly from (0.79 ± 0.03) at 15 min to (1.10 ± 0.02) at 2 h, and from (1.35 ± 0.06) at 4 h to (1.48 ± 0.02) at 6 h. There were significant differences in protein expression between the RNAi and the ischemic groups at all the time points (P <0.01) except at 15 min (P> 0.05). (Figure 8).

5. Correlation between AQP4 protein (D) and rADC value

The regression equation showed a negative correlation between AQP4 protein expression and the rADC value: $D = -68.90 \times \text{rADC} + 57.855$, $r = -0.806$ (P <0.01).
Fig. 0: In ischemic group DWI shows high signal at the right basal ganglia at 15 min after embolism. And the high signal area and its intensity increases rapidly at 1 h and 2 h, and rADC value decreases fast. T2FLAIR and T2WI show high signal 6 h after embolization. There is no significant difference of the high signal area between those two sequences and DWI.

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Fig. 0: DWI imaging: A-RNAi group, B-control group, C- normal group: Group B shows high signal in the right basal ganglia at 15 min after embolism. And the scope and intensity of the high signal area increase rapidly within 2 h while rADC value decreases rapidly during this period. In group A rADC value shows no significant difference at 15 min (P > 0.05). High signal scope shows no significant increase at 1 h and 2 h, and the rADC value decreases slightly with a significant difference compared with group B (P
Fig. 0: The curve of ADC value: A-RNAi group, B-control group, C-normal group: Group B shows high signal in the right basal ganglia at 15 min after embolism. And the scope and intensity of the high signal area increase rapidly within 2 h while rADC value decreases rapidly during this period. In group A rADC value shows no significant difference at 15 min (P> 0.05). High signal scope shows no significant increase at 1 h and 2 h, and the rADC value decreases slightly with a significant difference compared with group B (P

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**Fig. 0:** Astrocytic swelling (#) and widened pericellular interspace indicates intracellular edema in the ischemic group at 15 min (HE × 400).

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Fig. 0: Mitochondria swelling (#) and vacuole (#) indicates intracellular edema in the ischemic group at 15 min (electron microscopy × 6 000).

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Fig. 0: In the RNAi group#B#, mitochondrial swelling and vacuole (glial cell edema) significantly reduceds compared with control group at 2 h#A# (electron microscopy × 6 000)

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Fig. 0: There were significant differences between the RNAi and ischemic group expression levels at each time point ($t = 17.20$, $P < 0.05$).

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Fig. 0: There were significant differences in protein expression between the RNAi and the ischemic groups at all the time points ($P < 0.05$).

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Conclusion

The up-regulating of the AQP4 expression should be the molecular mechanism of the cell edema, as well as, be resulting in the decreasing of the rADC. AQP4 RANi could inhibite the expression of AQP4 and mitigate cerebral cell edema effectively. But it become ineffective during the stage in which the angioedema is the mostly pathological change (4#6 h). The DWI could provide timely and exactly imaging information for diagnostic cerebral cell edema.
References


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